

## Separation of soil Organic Debris using Sucrose-ZnCl<sub>2</sub> Density Gradient Centrifugation

Seok Ho Jung, Doug Young Chung<sup>1</sup>, and Gwang Hyun Han\*

*Department of Environmental & Biological Chemistry, Chungbuk National University, Cheongju 361-763, Korea*

<sup>1</sup>*Department of Bioenvironmental Chemistry, Chungnam National University, Daejeon 305-764, Korea*

The active fraction of soil organic matter, which includes organic debris and light organic fraction, plays a major role in nutrient cycling. In addition, particulate organic matter is a valuable index of labile soil organic matter and can reflect differences in various soil behaviors. Since soil organic matter bound to soil mineral particles has its density lower than soil minerals, we partitioned soil organic matter into debris (< 1.5 g cm<sup>-3</sup>), light fraction (1.5-2.0 g cm<sup>-3</sup>), and heavy fraction (> 2.0 g cm<sup>-3</sup>), based on high density ZnCl<sub>2</sub>- sucrose solutions. Generally, partitioned organic bands were clearly separated, demonstrating that the ZnCl<sub>2</sub>- sucrose solutions are useful for such a density gradient centrifugation. The available gradient ranges from 1.2 to 2.0 g cm<sup>-3</sup>. Although there was not a statistically meaningful difference in organic debris and organomineral fractions among the examined soils, there was a general trend that a higher content of organic debris resulted in a higher proportion of light organomineral fraction. In addition, high clay content was associated with increased fraction of light organomineals. Partitioning of soil organic carbon revealed that carbon content is reduced in the heavy fraction than in the light fraction, reflecting that the light fraction contains more fresh and abundant carbon than the passive resistant fraction. It was also found that carbon contents in the overall organic matter, debris, light fraction, and heavy fractions may differ considerably in response to different farming practices.

**Key words:** Carbon content; Nutrient; Humus; Biomass; Organic matter fractionation

### Introduction

Recently, concerns over global warming (or climate change) have led to increasing interests in carbon stored in soils. On a global scale, about twice as much carbon is to be stored in the soil than in the world's vegetation an atmosphere combined (Batjes, 1996; Brady and Weil, 2004). The total carbon in soils is the sum of both organic and inorganic carbons. Organic carbon is present in the soil organic matter fraction, whereas inorganic carbon is mainly found in carbonate minerals (mostly in alkaline soils). Therefore, in many cases, the organic carbon contributes to most of the total carbon content of the soil (Eswaran et al., 1993). However, organic carbon contained in the soil organic fraction consists of very complex forms of carbon, which include cells of living microorganisms, plant and animal residue at various

stages of decomposition, and stable humus synthesized from residues (Nelson and Sommer, 1996).

It is generally considered that soil humic substances, which are huge molecules with variable complex structures, comprise 60 to 80% of the soil organic matter (Chen and Aviad, 1990). This humic fraction is resistant to microbial decomposition and exerts a major influence on cation exchange capacity, water holding capacity, and other many important soil properties. The rest 20 to 40% of soil organic carbon, which is less resistant to microbial decompositions, consists of living biomass (intact plant and animal tissues and soil microorganisms), plant or animal residues, and easily decomposable biomolecules (like as polysaccharides and polypeptides). This active fraction of soil organic matter plays a major role in nutrient cycling and various biochemical behaviors of soil (Brady and Weil, 2004).

In spite of the complexity of soil organic matter, plant residues (or organic debris) are considered to be the primary source of soil organic matter. Decomposition of

organic debris is controlled by various environmental factors and by the chemical compositions of the debris. Generally, high contents of lignin and polyphenols, along with high C/N ratios, considerably slow the decomposition process (Paustian et al., 1992).

Standard method for quantifying amounts of organic debris in soils has not been established yet. In many cases of preparation of soil samples for chemical analyses, organic debris is considered to be removed beforehand, but not to be quantified. One common method for removal of organic debris is floating residues over a saturated NaCl solution (Wolf et al., 1994), of which the density is about  $1.2 \text{ g cm}^{-3}$ . The soil is stirred vigorously, and then particulate organic debris floats to the surface. However, since a significant proportion of organic debris can have its density higher than  $1.2 \text{ g cm}^{-3}$ , it is not easy to remove (or collect) all of the organic debris. However, it is generally accepted that the floating and/or acid pretreatment for eliminating plant residue and carbonate have no effect on either the organic carbon content of the soil, or on the stable carbon composition of the soil organic matter (Wolf et al., 1994).

On the other hand, density fractionation of soil organic matter has been utilized to study behaviors of organic matter that are associated with degree of decomposition and organic particulates (Swift, 1996). A number of methods have been developed to separate soil particles on the basis of their density in water (Turckenek and Oades 1979), and in organic liquids or salt solutions of high specific gravity (e.g., Roth et al., 1992; Baldock et al., 1990; Soilins et al., 1984). In those applications, expensive salts, such as cesium chloride and polytungstate solutions, are required and must be combined with particle size fractionation (e.g., sieving or sedimentation). Therefore, it is highly recommended to develop an efficient density fractionation method that requires less expensive chemicals but provides higher specific gravity solution.

The objective of the present study were (1) investigation of a simple density fractionation method based on highly soluble  $\text{ZnCl}_2$  and sucrose, (2) assessment of its application to quantification of soil organic debris, and (3) partitioning of soil organic matter based on the density of the component particles.

## Materials and Methods

**High specific gravity solutions** We examined densities of following solutions (or liquid) of various salts and salt-sucrose combinations: (1) saturated NaCl, (2) saturated sucrose (Daejung, CN 7501-4400, Korea), (3) saturated  $\text{ZnCl}_2$  (Samchun, PN Z0038, Korea), (4) saturated CsCl, (5) saturated sucrose-NaCl, (6) saturated sucrose- $\text{ZnCl}_2$  (up to  $1.6 \text{ g cm}^{-3}$ ), and (7) chloroform. The saturated salt solutions were prepared by dissolving the corresponding salt using a microwave oven (MM-M301, LG, Seoul, Korea). We tried an initial heating time of 3 min, with subsequent 2-min heatings until most of the salt dissolved and the solution turned clear to a certain extent. Complete dissolution of the salts was done by stirring the solution at room temperature. The approximate density of the solution was determined by measuring its volume with a graded cylinder. In case of the salt-sucrose combinations, the salts were dissolved in advance to dissolving sucrose. Sucrose was added to the solution stepwise by 50 g. The color of salt-sucrose solution became darker as the amount of sucrose or salt increased (pale brown in case of  $1.6 \text{ g cm}^{-3}$  sucrose- $\text{ZnCl}_2$  solution).

**Soil sampling and characterization** Soil samples were collected from 6 garlic fields in Danyang, Korea ( $\text{N}37^{\circ}07'$ ,  $\text{E}128^{\circ}20'$ ) in late June, 2011. The garlic fields were chosen to maximize the difference in soil organic matter content and in dry bulk density across the study area. Triplicate samplings of top 20-cm soil were done in each field using a core sampler (10-cm inner diameter, 20-cm long; made of PVC). The whole garlic bulb and rhizosphere soil were collected with the sampler. The size and volume of the bulb were determined using a graded cylinder in the field. After collection, soil samples were sealed in polyethylene bags and returned to the laboratory. Additional soil samples were collected similarly from a Chinese cabbage field in Cheongju, Korea ( $\text{N}37^{\circ}06'$ ,  $\text{E}127^{\circ}05'$ ) in mid November, 2011. Dry bulk densities of the soils were determined from the dry weight of the soil and the volume of the bulb. For physicochemical characterization of the soils, samples were air dried and ground to pass a sieve with 2-mm opening. Particle size analyses of samples were done by the pipette method (Gee and Bauder, 1986). Soil pH and EC were measured in water with a soil-to-water ratio

of 1:5 after shaking the mixture for 30 min (Thomas, 1996).

**Soil organic carbon content** Organic matter content was determined by the Walkley-Black method (Nelson and Sommer, 1996), after grinding the air-dried sample to pass through a 0.5-mm sieve. Samples are considered to contain a significant proportion of organic debris and light organominerals. To stimulate the acidic digestion process to complete, external heating with a microwave oven in a fume hood was carried out for 2 min, right after adding  $K_2Cr_2O_7$  and  $H_2SO_4$  into the sample flask. After then, the flask was allowed to stand on an insulated sheet for about 30 min. It was previously known that the dichromate method with little (or no) external heating gives very poor recovery of soil organic carbon (Nelson and Sommer, 1996).

**Fractionation of soil organic matter** We partitioned soil organic matter into debris ( $< 1.5 \text{ g cm}^{-3}$ ), light fraction ( $1.5\text{-}2.0 \text{ g cm}^{-3}$ ), and heavy fraction ( $> 2.0 \text{ g cm}^{-3}$ ), on the basis of the density of the component particles. When soil organic matter is tightly bound to soil mineral particles, the resulting organic-mineral complex would have its density lower than soil minerals, but higher than organic particulates (Swift, 1996). In the previous studies of Baldock et al. (1990) and, Marriott and Wander (2006), sodium polytungstate ( $Na_3WO_4 \cdot 9WO_3 \cdot H_2O$ ) solution was used as the high specific gravity bed. However, in the present study, we prepared the high-density solutions from saturated sucrose- $ZnCl_2$  ( $1.5 \text{ g cm}^{-3}$ ; 100 ml of water + 75 g of  $ZnCl_2$  + 195 g of sucrose) or concentrated  $ZnCl_2$  solutions ( $2.0 \text{ g cm}^{-3}$ ; 100 ml of water + 380 g of  $ZnCl_2$ ).

Initially, air-dried soil ( $< 2 \text{ mm}$ ) including small organic debris was ground to pass a 0.5-mm sieve. While preparing air-dried soil, recognizable organic material ( $> 2\text{mm}$ ) that passed through the sieve has been removed by hand, as reported previously by Marriott and Wander (2006). A discontinuous density gradient was prepared by layering 10 ml of concentrated  $ZnCl_2$  solution ( $1.5 \text{ g cm}^{-3}$ ) and 10 ml of saturated sucrose- $ZnCl_2$  ( $1.5 \text{ g cm}^{-3}$ ) in a 50-ml centrifugation tube (polypropylene). Soil suspension, made of approximately 5.0 g of soil ( $< 0.5 \text{ mm}$ ) and 10 ml of water, was loaded onto the specific gravity solutions. To disintegrate the aggregates and to disperse all the colloidal particles,

the tube was ultrasonicated at  $30^\circ\text{C}$  and at a medium output for 30 min. During the sonication process, no appreciable disturbance of the density gradient was observed. Then, the sample was centrifugated for 10 min at 3000 rpm using a swing-rotor centrifuge (FLETA-5, Hani, Incheon, Korea). Immediately after centrifugation, the debris, and light and heavy organic fractions were carefully transferred into Buchner funnels and filtrated through a Whatman No 42 paper. The collected organic fractions were rinsed with about 50 ml of deionized water and dried using a microwave oven (MM-M301, LG, Seoul, Korea). We tried an initial heating time of 2 min, with subsequent 30-s heatings until dryness. The collected heavy organic fractions were then analyzed for their carbon contents using the Walkley-Black method as described above.

## Results and Discussion

### High specific gravity solutions based on $ZnCl_2$

Density gradients based on high gravity solutions are used in many different operations including separation particles of different densities (e.g., CsCl isopycnotography and sucrose zonal centrifugation). Especially, by fractionating the soil based on the density of the component particles (Swift, 1996), it is possible to partition the organic matter into fractions of different physical characteristics (e.g., particle size distribution and their weigh fractions) as well as different chemical behaviors (e.g., relative degree of decomposition). To achieve this kind of fractionation, utilization of solutions with high specific gravity is necessary.

Table 1 lists the solubilities and maximum solution densities of the examined solutes. NaCl and sucrose solutions are cheap and easy to handle. However, their

**Table 1. Solubilities and maximum solution densities of the examined solutes and solvent.**

Chemical	Solubility at	Maximum Solution
	25°C	Density
	----- $\text{g L}^{-1}$ -----	----- $\text{g cm}^{-3}$ -----
NaCl	359	1.2
Sucrose	2000	1.3
$ZnCl_2$	4320	2.2
CsCl	1865	1.8
$CHCl_3$	$8^\dagger$	1.5

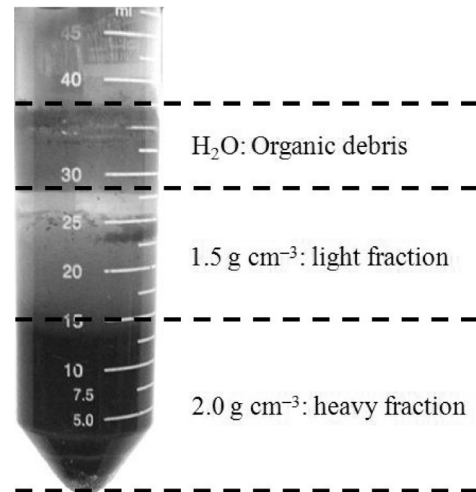
$^\dagger$  at  $20^\circ\text{C}$

**Table 2. High specific gravity solutions based on ZnCl<sub>2</sub> and sucrose.**

Amount of solute in 100 ml water		Solution density
ZnCl <sub>2</sub>	Sucrose	
----- g -----	----- g -----	g cm <sup>-3</sup>
-	100	1.22
-	110	1.20
-	130	1.26
-	150	1.25
-	195 (Saturated)	1.30
50	195 (Saturated)	1.40
100	195 (Saturated)	1.60
380	-	2.00

solution densities are lower (at maximum 1.3 g cm<sup>-3</sup>) than those required for optimum fractionation of soil organic matter. In many cases, a sodium polytungstate-based solution of 1.6 to 2.0 g cm<sup>-3</sup> is used for separation of light and heavy organic fractions (Baldock et al., 1990; Marriott and Wander, 2006). Using a dense organic liquid such as chloroform can provide a moderate density. However, it can cause the health, safety, and environmental problems, in addition to considerable extraction of important organic fractions (Swift, 1996). CsCl is one of favorite salts (e.g. for equilibrium density gradient centrifugation of DNA), but quite expensive. Commonly employed gradients has specific gravities from 1.1 (top) to 1.8 (bottom). On the other hand, ZnCl<sub>2</sub>, which is available at a low price, has a quite high solubility in water and subsequently high solution density. Table 2 lists high specific gravity solutions that can be obtained from variable combinations of ZnCl<sub>2</sub> and sucrose. The available gradient ranges from 1.2 to 2.0 g cm<sup>-3</sup>. Like as in the conventional sucrose zone centrifugation, various ZnCl<sub>2</sub>- sucrose combinations are expected to provide gravitational stability as different species move down tube at different rates with forming separate bands.

**Density gradient centrifugation** Fig. 1 depicts the bands of soil organic matter fractionated by the density gradient centrifugation. Since soil organic matter bound to soil mineral particles has its density lower than soil minerals, we partitioned soil organic matter into debris (< 1.5 g cm<sup>-3</sup>), light fraction (1.5-2.0 g cm<sup>-3</sup>), and heavy fraction (> 2.0 g cm<sup>-3</sup>), on the basis of the density of the component particles. Commonly, particle density of



**Fig. 1. Density gradient centrifugation for separation of soil organic fractions. A discontinuous density gradient was prepared by layering 10 ml of concentrated ZnCl<sub>2</sub> solution (1.5 g cm<sup>-3</sup>) and 10 ml of saturated sucrose-ZnCl<sub>2</sub> (1.5 g cm<sup>-3</sup>) in a 50-ml centrifugation tube.**

2.0 g cm<sup>-3</sup> is used to divide the light and heavy organic fractions (Baldock et al., 1990; Marriott and Wander, 2006). Generally, partitioned organic bands were clearly separated and demonstrated that the ZnCl<sub>2</sub>- sucrose solutions are useful for such a density gradient centrifugation. Many previous studies have shown that particulate organic matter is a valuable index of labile soil organic matter and can reflect differences in land management and soil properties (e.g., Golchin et al., 1994; Carter 2002). Density fractionation is free from solvent extraction and considered to be capable of separating the soil organic matter according to age, with the youngest (most fresh) fraction being the lightest (Swift, 1996). The results in the present study suggest that it is possible to partition the organic matter into multiple fractions with the ZnCl<sub>2</sub>- sucrose solutions.

**Density fractionation of soil organic matter** Table 3 compares the physicochemical properties of the examined soils with their organomineral fractions (organic matter bound to soil minerals). Since recognizable organic material (> 2mm) that passed through the sieve has been removed by hand while preparing air-dried soil, the proportion of organic proportion was not significant in the examined soils. Although there was not a statistically meaningful difference in organic debris and organomineral fractions among the examined soils, general trend was shown that a higher content of organic debris resulted in a higher proportion of light

**Table 3. Physicochemical properties and fractions of organominerals.**

Crop field <sup>†</sup>	Db	pH	EC	Clay	Organic Debris	Light organominerals	Heavy organominerals
	g cm <sup>-3</sup>		dS/m	%	g kg <sup>-1</sup> (average ± SD)		
GF-A	1.2	8.4	0.23	20	3.9 ± 0.5	8.4 ± 2.2	987.7 ± 2.6
GF-B	1.3	7.2	0.14	43	6.7 ± 3.2	9.0 ± 2.2	984.3 ± 1.3
CC	1.1	6.7	0.05	24	3.7 ± 1.6	7.2 ± 0.5	989.1 ± 1.5

<sup>†</sup>GF-A: garlic field A, GF-B: garlic field, CC: Chinese cabbage field.

**Table 4. Carbon percentages of bulk soils and heavy organominerals.**

Crop field <sup>†</sup>	OM	Heavy organic fraction
	%	
GF-A	2.67 ± 0.05	2.62 ± 0.34
GF-B	3.41 ± 0.01	3.08 ± 0.04
CC	1.95 ± 0.04	1.54 ± 0.15

<sup>†</sup>GF-A: garlic filed A, GF-B: garlic filed B, CC: Chinese cabbage field

**Table 5. Carbon contents in soil organic fractions.**

Crop field <sup>†</sup>	OM	Debris <sup>‡</sup>	Light organic fraction <sup>§</sup>	Heavy organic fraction
	g C kg <sup>-1</sup> soil			
GF-A	2670 ± 53	1.5 ± 0.2	81 ± 20	2587 ± 338
GF-B	3410 ± 18	2.6 ± 0.6	366 ± 91	3040 ± 45
CC	1950 ± 37	1.4 ± 1.0	420 ± 42	1528 ± 152

<sup>†</sup>GF-A: garlic filed A, GF-B: garlic filed B, CC: Chinese cabbage field.

<sup>‡</sup>The carbon contents were evaluated assuming C = org matter × 0.38 C fraction.

<sup>§</sup>The carbon contents were evaluated from the total C contents of total organic matter, debris, and heavy organic fraction.

organomineral fraction. This can be mainly attributed to the fact that plant residues (or organic debris) are the primary source of soil organic matter and they constitute the active portions of soil organic pool. In addition, we observed that high clay content was associated with increased fraction of light organominerals. Other physicochemical properties (dry bulk density, soil pH, and EC) were not related to difference in proportions of organomineral fractions. Many previous studies have shown that both the quantity and quality of labile soil organic matter are important for an appropriate land management. Especially, organic fractions that are sensitive to short-term changes in environmental and soil factors are important for developing fertility management tools for sustainable farming (Marriott and Wander, 2006).

**Partitioning of soil organic carbon** Table 4 compares carbon percentages between the total soil and the heavy organomineral fraction. In all the soils examined, a

significant reduction in carbon percentage was observed when organic carbon is fractionated in the heavy fraction. This indicates that the light fraction contains more fresh and abundant carbon than the passive resistant heavy fraction. The heavy fraction is considered to be more resistant to microbial decomposition and more passive in nutrient cycling than the light fraction (Brady and Weil, 2004).

Table 5 shows the amount of carbon stored in each soil organic fraction, which was estimated from the carbon percentages in Table 4 and the amount of organomineral fractions in Table 3. Between the garlic fields, soil with high organic matter content had more carbon in the debris, light fraction, and heavy fractions. In comparison to those garlic fields, soils from Chinese cabbage field had more carbon in the light fraction in spite of much lower organic matter content. This difference between crop fields may reflect that the quantity and quality of labile soil organic matter can vary significantly in response to different farming practices.

Marriott and Wander (2006) reported that the characteristics of particulate organic matter reflects how organic practices improve the quality of soil organic matter and the associated nutrient and substrate decay dynamics in the farming system.

Overall, although density gradient fractionation of organic matter is labor intensive and time-consuming, it can provide more detailed information on organic matter dynamics and nutrient cycling in the field. Therefore, it is highly recommend to assess labile soil organic fraction and the quality of these fractions for maximizing nutrient and water use efficiency of the production system.

### Conclusion

When soil organic matter is tightly bound to soil mineral particles, the resulting organic-mineral complex would have its density lower than soil minerals, but higher than organic particulates. We partitioned soil organic matter into debris, light fraction, and heavy fraction, based on the density of the component particles using dense sucrose-ZnCl<sub>2</sub> solutions. Generally, partitioned organic bands were clearly separated and demonstrated that the ZnCl<sub>2</sub>- sucrose solutions are useful for such a density gradient centrifugation. There was a general trend that a higher content of organic debris resulted in a higher proportion of light organomineral fraction, reflecting that plant residues are the primary source of soil organic matter. We also found that there can be a significant difference in the quantity and quality of organic fractions among crop fields managed by different farming practices.

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